

- (Amended) The method according to Claim 23, wherein [the gene encoding] said 6. [endogenous] wild-type extracellular protease gene has been deleted by homologous or illegitimate recombination.
- (Amended) The method according to Claim 23, wherein at least one copy of [a 7. plasmid comprises] said expression cassette is contained in a plasmid.
- 9. (Amended) The method according to Claim [7] 23, wherein said [mutant] mutated high alkaline protease gene is [obtained] derived from a Bacillus novo species PB92 high alkaline protease gene.
- (Amended) The method according to Claim 7 or 23, wherein at least one copy of 10. said expression cassette is integrated into the genome of said alkalophilic Bacillus strain host.

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(Amended) A method of obtaining [an] a non-reverting alkalophilic Bacillus 12. strain having no detectable wild-type extracellular high alkaline protease, said method comprising:

transforming an alkalophilic Bacillus strain containing a wild-type high alkaline protease gene endogenous to said alkalophilic Bacillus strain with a cloning vector comprising [the] 5' and the 3' flanking regions but not [the] a coding region of a [gene coding for the] high alkaline protease gene and DNA encoding a replication function, wherein a sufficient amount of said flanking regions is present to provide for homologous recombination with [an endogenous gene coding for the] said wild-type high alkaline protease gene whereby transformants having an inactivated wild-type high alkaline protease gene are obtained;

identifying said transformants by growing said transformants under growth conditions to which [the] said replication function [of said cloning vector] is sensitive, whereby [the] said replication function [encoded by said vector] is inactivated; and

isolating [said] transformants so identified [as having said inactivated replication function and] whereby a non-reverting alkalophilic Bacillus strain having no detectable wild-type extracellular high alkaline protease is obtained.



- 13. (Twice Amended) The method according to Claim 12, wherein said alkalophilic *Bacillus* strain is *Bacillus* novo species PB92 or a derivative thereof [which is incapable of reversion and contains a mutant high alkaline protease].
- 14. (Twice Amended) An alkalophilic *Bacillus* strain producing a [mutant] <u>mutated</u> high alkaline protease [which is free of expression product of an] <u>and no detectable</u> [indigenous] <u>wild-type</u> extracellular alkaline protease [gene] <u>endogenous to said alkalophilic *Bacillus* strain, wherein said strain [has been] <u>is</u> obtained by [transforming] <u>growing</u> an alkalophilic *Bacillus* strain [having no detectable indigenous extracellular high alkaline protease obtained by the method] according to Claim 12 or 13, [or 27 with] <u>transformed with</u> [a plasmid] <u>an</u> expression vector comprising [the] <u>a</u> [mutant] <u>mutated</u> high alkaline protease gene <u>under conditions whereby said mutated high alkaline protease gene is expressed</u>.</u>

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- 19. (Reiterated) A detergent composition comprising as an active ingredient at least one mutant form of high alkaline protease prepared according to the method of Claim 23.
- 23. (Amended) A method for [production of] <u>obtaining</u> a mutated high alkaline protease free of [endogenous] <u>wild-type</u> extracellular high alkaline protease, said method comprising:

isolating said mutated high alkaline protease from growth medium or a cell lysate of [growing] a[n] non-reverting alkalophilic Bacillus strain host [incapable of reversion and having] which produces no detectable [endogenous] wild-type extracellular high alkaline protease endogenous to said alkalophilic Bacillus strain host [as a result of deletion of the gene for endogenous extracellular protease transformed with], wherein said alkalophilic Bacillus strain host contains at least one copy of an expression cassette [providing for expression of said] comprising a [mutant] mutated high alkaline protease gene [in said host,] and wherein said alkalophilic Bacillus strain host is grown under conditions whereby said [mutant] mutated high alkaline protease gene is [produced; and isolating said mutant high alkaline protease] expressed.



- 24. (Reiterated) A method for preparing a detergent composition, which comprises the step of combining a detergent composition with, as an active ingredient, at least one mutant form of a high alkaline protease prepared according to the method of Claim 23.
- 25. (Reiterated) A method for processing laundry, which comprises the step of contacting said laundry with a detergent composition comprising as an active ingredient at least one mutant form of a high alkaline protease prepared according to the method of Claim 23.

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- 29. (Twice Amended) A[n] <u>non-reverting</u> alkalophilic *Bacillus* strain [producing] <u>which produces</u> a [mutant] <u>mutated</u> high alkaline protease, <u>wherein said strain</u> [which is incapable of reversion and which is free of expression product of an endogenous] <u>does not produce a wild type</u> extracellular <u>high</u> alkaline protease [gene] <u>endogenous to said alkalophilic</u> <u>Bacillus</u> strain.
- 30. (Twice Amended) A method for [production of] <u>obtaining</u> a mutated [high alkaline] <u>Bacillus</u> novo species <u>PB92</u> protease free of [endogenous] <u>wild-type</u> extracellular high alkaline protease, said method comprising:

isolating said [mutant high alkaline] mutated Bacillus novo species PB92 protease from [a culture broth] growth medium or cell lysate of a non-reverting alkalophilic Bacillus strain host [cells wherein said cells are substantially incapable of reversion and have] which produces no detectable [endogenous] wild-type extracellular high alkaline protease [as a result of deletion-of-the gene for] endogenous to said alkalophilic Bacillus strain [extracellular protease and wherein said cells produce a mutant high alkaline protease as a result of transformation of said cells or predecessor cells with] wherein said alkalophilic Bacillus strain host contains at least one copy of an expression cassette [providing for expression of said mutant high alkaline] comprising a mutated Bacillus novo species PB92 protease gene [in said host cells] and wherein said alkalophilic Bacillus strain host is grown under conditions whereby said mutated Bacillus novo species PB92 protease gene is expressed.

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- 31. (Amended) The method according to Claim 30, wherein [untransformed parent cells of] said alkalophilic *Bacillus* strain host [cells are] is a *Bacillus novo* species PB92 strain [cells] or a derivative species thereof.
- 32. (Amended) The method according to Claim 30 or 31, wherein [untransformed parent cells of] said alkalophilic *Bacillus* strain host [cells are] is asporogenic.
- 33. (Amended) The method according to Claim 30 wherein said <u>non-reverting</u> alkalophilic *Bacillus* strain host [cells are] <u>is</u> free of [untransformed parent] cells <u>which express a</u> wild type high alkaline protease gene endogenous to said alkalophilic *Bacillus* strain host.
- 34. (Amended) An alkalophilic *Bacillus* strain comprising a non-reverting <u>wild-type</u> extracellular <u>high alkaline</u> protease-negative phenotype, wherein said strain or an ancestor of said strain was stably transformed with an [exogenous protease gene encoding a mutant] <u>expression cassette comprising a high alkaline protease gene and wherein said strain has an increased efficiency in production of said mutant high alkaline protease as compared to an untransformed <u>alkalophilic *Bacillus*</u> strain of the same species.</u>
- 35. (Amended) The alkalophilic *Bacillus* strain according to Claim 34, wherein said phenotype is [the] <u>obtained as a</u> result of a deletion of a sufficient amount of [an endogenous] <u>a</u> <u>wild-type</u> extracellular protease <u>gene endogenous to said alkalophilic-Bacillus</u> strain so as to prevent reversion of said [non-reverting extracellular protease-negative] phenotype <u>to a wild-type</u> extracellular high alkaline protease-positive phenotype.

Cancel Claims 36-37.

Add the following new claims.

--38. (New) In detergent composition which has as an active ingredient at least one mutated high alkaline protease, the improvement which comprises no detectable wild-type high alkaline protease with said mutated high alkaline protease.

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